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Qualitative and Quantitative Phytochemical Analysis of *Centella asiatica*

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Abstract

Centella asiatica commonly known by names like gotu kola, Mandukaparni or Indian pennywort is a small herbaceous perennial medicinal plant belonging to *Apiaceae* family. Its active metabolites are triterpenoids which includes asiaticoside, asiatic acid, madecassoside, madecassic acid which has wide range of pharmaceutical activities like wound healing, memory enhancer, treatment of skin diseases etc. The present work is aimed to perform phytochemical analysis and estimation of antioxidant activity by DPPH assay. The antioxidant activity by DPPH assay showed maximum IC_{50} value in CSC extract than shoot and callus extracts. The quantitative estimation of total phenolic, flavanoids and tannins indicates highest phytochemicals in SC when compared to CC and CSC.

Keywords: *Centella asiatica*; Shoot culture; DPPH; Total phenolic content; Total flavanoid contents; Total tannins contents

Introduction

Centella asiatica, a small edible, herbaceous medicinal plant belonging to *Apiaceae* family native to India, Sri Lanka, Iran, New Guinea, Australia, Indonesia southern and central Africa [1]. This plant is listed as an important drug in the Indian Herbal Pharmacopoeia, European Pharmacopoeia, Pharmacopoeia of the People's Republic of China and German Homoeopathic Pharmacopoeia [2]. In 2006, it was ranked third position in a priority list of most essential Indian medicinal plants based on their pharmaceutical and economic importance and also the demands for its raw material is constantly rising throughout the world [3]. *Centella* contains several valuable secondary compounds or terpenoids, known as centelloids, which includes asiaticoside, madecassoside, centelloside, centellose, brahminoside, thankunizide, sceffoleoside, brahmoside, and asiatic, centellic, brahmic, and madecassic acids [4-6]. The most biologically active compounds include triterpene saponin like asiatic acid, asiaticoside, madecassoside and madecassic acid, which are responsible for a wide range of therapeutic activity [7-9].

Antioxidant is any substance which prevents or retards deterioration richly available in fruits and green leafy vegetables which is known to reduce the risk of cancer, cardiovascular diseases. Some antioxidant components are ascorbic acid, carotenoids, vitamin E, polyphenols and other phytochemicals. A free radical is substance with unpaired electrons causes damage to lipids, protein, enzyme thus antioxidants prevents these damages by neutralizing them. Phytochemical imparts health benefits beyond basic nutrition [10]. Several plant extracts and different class of phytochemicals are known to have antioxidant activity [11]. Flavonoids are potent antioxidant and its capacity depends on their molecular structure and Quercetin is one the most abundant dietary flavonol usually used as standards. It was also reported that plant contains tannins, sugars, inorganic acids [12], amino acids like phenylalanine, aspartic acid, α alanine, glutamic acid and glycine [13]. The antioxidant activity of *Centella* plays a key role in acting against the reactive oxygen species in our body [14]. Due to health benefits like antioxidant activity, the usage of *Centella* in food and beverages has been increased over these years. In the present study qualitative phytochemical analysis of *Centella asiatica* was done in three different culture system i.e., shoot, callus and cell suspension culture. Further antioxidant activity, total phenolic, total flavonoid and total tannin content were estimated.

Materials and Methods

Shoot culture

Air dried plant material (0.5 g) was weighed and crushed to fine powder and then add 5 ml of 80% methanol was added and mixed properly. Sonicate for 15 minutes and left at room temperature for 24 hrs. The extract was centrifuged for 10 minutes at 1500 g and the supernatant was collected and stored at 4°C until further use.

Callus culture

For callus extracts, approximate 0.5 g of air dried callus was soaked in 5 ml of 80% methanol and mixed properly, then sonicate for 15 minutes and centrifuge for 10 minutes at 1500 g and the supernatant was collected and stored at 4°C until further use.

Cell suspension culture

For Cell suspension cells extract, the cell suspension was transferred into sterile falcon tubes and centrifuged at 1500 g for 15 minutes and then the supernatant was removed so that cells remains at the bottom as pellet. Approximate 0.5 g of cells was weighed and adds 5 ml of 80% methanol mix it properly. Then sonicate the samples for 10 minutes. Again, centrifuged the samples at 1500 g for 10 minutes and the supernatant was collected and stored at 4°C until further use.

Preliminary screening of phytochemicals

a) Test for Terpenoids (Salkowski test): About 5 ml of sample was mixed with 2 ml of chloroform in a test tube to which 3 ml of concentrated sulphuric acid was carefully added through the sides to

form a layer. If reddish brown colour appears at the interface, indicates presence of terpenoids.

b) Test for Saponin: In a test tube, few amount of sample was taken and mixed properly with 5 ml of distilled water and was vigorously shaken, if stable foam appears, then it indicates presence of saponin.

c) Test for Tannins: In a test tube, about 1 ml of the sample was taken and then add 1 ml of Potassium ferricyanide (0.008 M) then 1 ml of Ferric chloride (0.02 M) containing 0.1 N HCl was added. Blue-black coloration indicates the presence of tannin.

d) Test for Alkaloids: In a test tube few quantity of sample is mixed with 2 ml of Wagner's reagent (2 g of iodine and 6 g of potassium iodide in 100 ml distilled water). If reddish brown colored precipitate is formed indicates the presence of alkaloids.

e) Test for Flavonoids: In a test tube, about 5 ml of dilute ammonia solution was added to small amount of the sample followed by addition of concentrated sulphuric acid. A yellow colour indicates the presence of flavonoids which disappears after some time.

f) Test for Steroids: In a test tube, 1 ml of the sample was taken and dissolved in 5 ml chloroform, then equal volume (5 ml) of concentrated sulphuric acid was carefully added through the sides of test tube. If upper layer turns into red colour and the sulphuric acid layer turns yellow colour with slight green fluorescence which indicates the presence of steroid.

Antioxidant activity by DPPH free radical assay

Sample stock solutions of 1 mg/ml concentration prepared which were diluted with methanol to final concentrations of 5, 10, 25, 50, 125, 250, µg/mL to make a total volume of 2.5 ml. 1 mL of a 0.3 mM DPPH prepared methanol solution was added to different concentrations of sample solutions and allowed to react at room temperature. Blank was methanol (1.0 mL) and plant extract solution (2.5 mL). The negative control was DPPH solution (1.0 mL) and methanol (2.5 mL). The positive controls are the standard solutions which is ascorbic acid for which all the above procedure was repeated. After 30 min the absorbance values of standard and samples were measured at 518 nm and converted into the antioxidant activity in percentage using the following formula:

Antioxidant activity (%)=100-{[(Absorbance of sample-Absorbance of blank})*100]/Absorbance of control}

The I_0 value was calculated from the plots where the X axis represents the concentration of sample/standard and the Y axis is the percentage of antioxidant activity.

Experiment was performed in triplicates

Flavanoid estimation: The Aluminium chloride colorimetric assay was done to estimate flavanoids. Take 1 ml of sample and mix it with 4 ml of distilled water in a test tube. Then 0.3 ml of prepared sodium nitrite (5%) was added. 5 minutes later, 0.3 ml of prepared aluminium chloride (10%) was mixed properly. After 5 minutes, 2 ml of prepared sodium hydroxide (1 M) was added and made upto to 10 ml using distilled water. A standard solution of quercetin of concentration 20, 40, 60, 80 and 100 µg/ml was prepared using the above procedure. The absorbance of the sample and qurecetin standards was determined using an UV or Visible spectrophotometer at 510 nm. The total flavonoid content obtained was expressed in terms of mg of Qurecetin Extract per gram of extract.

Phenol estimation: The Total phenol content was estimated using Folin-Ciocalteu (F-C) reagent method. Firstly F-C reagent (10%) was prepared by 10 ml of F-C reagent added to 90 ml in water. Then sodium carbonate (5%) was prepared by dissolving 3 g of sodium carbonate in 50 ml of distilled water. Then in a test tube take about 200 µL sample to which 1.5 ml of prepared F-C reagent was added and kept at the dark condition for 5 minutes. After which, 1.5 ml of prepared sodium carbonate was added and then mix it properly. All the test tubes were again kept in the dark at dark conditions for about 2 hours.

The standard used was Gallic acid whose calibration curve was plotted. About 3 mg of Gallic acid was taken and dissolve it in 10 ml of methanol to get 300 mg/L concentration. Similarly 200, 100, 50 and 25 mg/L concentrations were prepared. Then the above mentioned procedure was followed for all the standard concentrations. The absorbance of all the samples and standards was determined using UV spectrophotometer at a wavelength of 750 nm.

Tannin estimation: The tannin content was estimated by Folin-Ciocalteu method. About 0.1 ml of the sample extract was treated with 7.5 ml of distilled water, with 0.5 ml of prepared F-C reagent (10%) reagent and adds 1 ml of the prepared sodium carbonate solution (35%) and then dilutes it to 10 ml using distilled water. The entire reaction mixture was mixed well and kept at room temperature for 30 min. The gallic acid was used as the standard and prepared to various concentrations like 20, 40, 60, 80 and 100 µg/ml with methanol. Finally, the absorbance of all the samples and gallic acid standards were determined using an UV or Visible spectrophotometer at 725 nm. The tannin content obtained was expressed as mg of gallic acid equivalent (GAE) per gram of extract

Results and Discussion

Preliminary phytochemical screening

When preliminary phytochemical screening was done in shoot, callus and cell suspension extracts, the positive results were obtained in tannins, flavanoids, terpenoid, saponin and steroids in all the three cultures and absence of alkaloids. Similar report has been obtained by Roy and Bharadvaja [15] in case of *Centella asiatica* shoot culture (Table 1).

Phytochemicals	Plant extract	Callus extract	Cell suspension cells extract
Alkaloids	-	-	-
Tannin	+	+	+
Flavanoids	+	+	+
Terpenoids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
+ for positive result - for negative result			

Table 1: Preliminary Phytochemical screening of methanolic extracts of *Centella asiatica*.

Antioxidant activity

IC_{50} value was calculated from the %inhibition vs. concentration graph which indicates the concentration of sample that is required for scavenging 50% of DPPH free radical. The IC_{50} value was highest of 21.25 µg/ml for cell suspension culture followed by shoot culture 19 µg/ml and then callus extract with 10 µg/ml. The ascorbic acid was taken as the standard with the IC_{50} value of 26.25 µg/ml. It was reported that ultrasonic assisted extraction showed highest recovery and highest antioxidant activity with 79% scavenging activity [16]. The IC_{50} values reported in *C. asiatica* with 100% ethanol extracts is 35.6 ± 1.3 µg/ml, 50% ethanol extract is 7.1 ± 1.5 µg/ml of and water extract is 10.3 ± 1.2 µg/ml [17] (Table 2 and Figure 1).

Samples	IC_{50} value (µg/ml)
Plant extract	19 ± 0.83
Callus extract	10 ± 0.79
Cell suspension sample	21.25 ± 1.2
Ascorbic acid(standard)	26.25 ± 0.64

Table 2: The IC_{50} values calculated from the graphs.

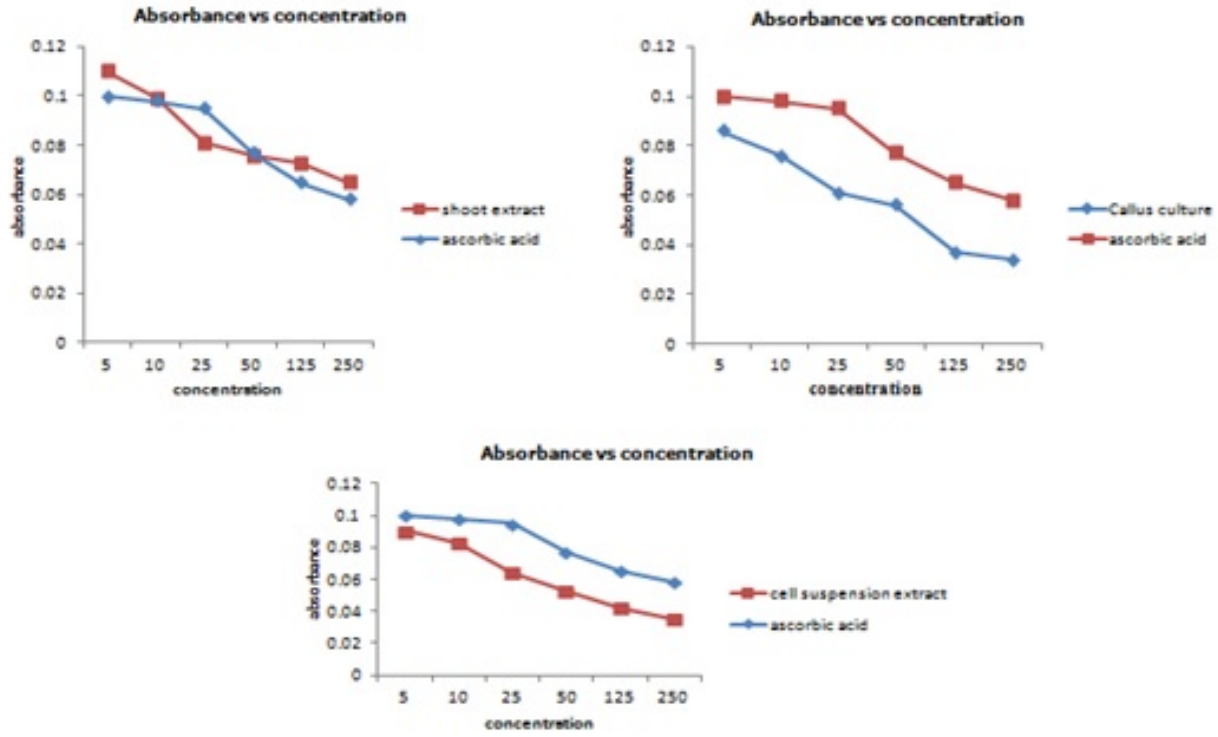

Figure 1: Absorbance vs concentration graph of ascorbic acid and a) shoot extract, b) Callus extract, c) Cell suspension extract.

Total phenolic, flavonoid and tannin estimation

The polyphenol content expressed in terms of mg of gallic acid equivalent per gram of extract was found to be highest in plant extract of 372.5 mg of GAE/g of extract followed by callus extracts having 182.5 mg of GAE/g of extract and least in cell suspension extract, 70 mg of GAE/g of extract. The total flavanoid estimation indicated in terms of qurecetin equivalent per gram of extract with maximum value of 275 ± 0.8 of mg QE/g of extract, following callus 145 ± 0.4 mg QE/g of extract and 100 ± 0.7 QE/g of extract in cell suspension cells. The Total tannin content estimate using gallic acid as standard found to maximum in shoot extract, 260 ± 0.55 mg of GAE/g of extract, followed by callus, 205 ± 0.7155 mg of GAE/g of extract and 55 ± 0.8 mg of GAE/g of extract in cell suspension extracts. According to reports, *Centella* extracts by ultrasonic assisted extraction showed Total Phenolic Content of 1350 mg GAE/100 g Dry Weight and Total Flavonoid Content, 599 mg CE/100 g Dry weight [16]. The polyphenols in 100% ethanol extract was 21.1 ± 0.1 Pyrogallol Equivalent and flavanoid is 9.3 ± 0.3 Quercetin Equivalent [17]. The polyphenols in *Centella* was found to be 150 mg tannic acid/100 g for *C. asiatica* [18]. The total antioxidant capacity was found to be very less in *C. asiatica* (623.78 µmol of ascorbic acid/g of sample). The IC_{50} value was estimated to be 19.89 mg/ml for *Centella asiatica* (Table 3) [19].

S No.	Samples	Total phenolic content (mg/g)*	Total flavanoid estimation (mg/g)	Total tannin content(mg/g)*
1	Shoot extracts	372.5	275 ± 0.8	260 ± 0.55
2	Callus extracts	182.5	145 ± 0.4	205 ± 0.71
3	Cell suspension cells extracts	70	100 ± 0.7	55 ± 0.8
*Expressed in terms of mg of gallic acid equivalent per gram of extract.				

Table 3: Total phenolic, flavanoid and tannin content in Shoot, Callus and Cell suspension extracts.

Conclusion

Qualitative phytochemical analysis showed the presence of flavanoids, tannins, terpenoid, saponin and steroids in all the three culture systems whereas absence of alkaloids. Antioxidant activity was maximum in cell suspension culture i.e. 21.25 µg/ml. Highest amount of phenolic, flavanoid and tannin content was present in the shoot culture.

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